

What is Claimed is:

1. A method for detecting an interaction between a first test protein and a second test protein at variable sensitivities, the method comprising:

(a) providing a host cell, wherein the host cell comprises a detectable reporter gene capable of expressing a detectable reporter gene product;

(b) providing to the host cell a first interacting molecule, which may be a macromolecule or a small molecule, comprising a region capable of binding DNA and a second interacting molecule, which may be a macromolecule or a small molecule, comprising a region capable of transcriptional activation, wherein the host cell is additionally provided with the capacity to regulate the absolute or relative amounts of the first or second macromolecules or small molecules;

(c) regulating the amounts of the first or second macromolecules or small molecules so the detectable reporter gene is activated; and

(d) determining the extent to which the detectable reporter gene has been activated.

2. The method of Claim 1, wherein the activation of the detectable reporter gene results in production of a detectable reporter protein.

3. The method of Claim 1, wherein the first or second interacting molecule is a protein, a DNA, a RNA, a nucleic acid, another macromolecule, a small molecule, a pharmaceutical agent, or another biologically or chemically interacting molecule.

See 99 → 4. The method of Claim 3, wherein the first or second interacting molecule is a macromolecule and may be a protein, a DNA, or a RNA provided by introducing into the host cell a first or second chimeric gene capable of being transcribed in the host cell.

5. The method of Claim 4, wherein the first chimeric gene comprises a first exogenously activatable promoter, a sequence coding for a DNA binding region or polypeptide, and a sequence coding for the macromolecule.

6. The method of Claim 5, wherein the first exogenously activatable promoter is activated by a first exogenous activator.

7. The method of Claim 4, wherein the first interacting molecule is provided by introducing into the host cell with a second chimeric gene capable of being transcribed in the host cell.

8. The method of Claim 7, wherein the second chimeric gene comprises a second exogenously activatable promoter, a sequence coding for a transcriptional activation domain or polypeptide, and a sequence coding for the second macromolecule.

9. The method of Claim 8, wherein the second exogenously activated promoter is activated by a second exogenous activator.

10. The method of Claim 6 or Claim 9 wherein at least one of first or second exogenous activators is a natural or synthetic, metabolically active or inactive steroid, steroid analogue, or steroid mimic.

11. The method of Claim 10, wherein at least one of the first or second exogenous activators is chosen from the group consisting of cortisol, hydrocortisone, mineralocorticoid, estrogen, estradiol, estrone, progesterone, androgen, ecdysone, retinoid, steroid complementary to orphan receptors, mineralocorticoid and mineralocorticoid analogue, or other agents capable of interacting with steroid responsive elements.

12. The method of Claim 1, additionally comprising rendering the host cells capable of regulating the relative or total amounts of the first or second interacting molecules or other macromolecules or small molecules in response to a modulatory agent acting at one or more of an extracellular, membrane, intracellular or nuclear site in order to give at least one of a continuous or discontinuous adjustment of a selected reporter sensitivity, wherein the modulatory agent consists of at least one of:

- (a) a natural or synthetic, metabolically active or inactive steroid, steroid analogue or steroid mimic, including glucocorticoids, dexamethasone, cortisone, cortisol, hydrocortisone, mineralocorticoids, estrogens, estradiol, estrone, progestones, androgens, ecdysones, retinoids, and steroids complementary to orphan receptors, mineralocorticoid or mineralocorticoid analogue, or other agent interacting with steroid responsive elements;
- (b) a membrane-active agent or analogue thereof, including an ionophore, anesthetic agent, detergent, amphoteric agent, hydrophobic agent, lipid-active agent, solvent, transmembrane signaling agent, intramembrane signaling agent, and farnesylating agent;
- (c) a small molecular pharmaceutical agent, including an antimicrobial agent, anti-tumor agent, nucleic-acid binding agent, cytoskeletal active agent, chelator, inducer, co-repressor, and agents affecting intracellular trafficking.

localization, protection and degradation of exogenous or endogenous mediators, hormones and molecules;

- (d) a biomolecule or natural or synthetic biopharmaceutical, including growth factors, cytokines, hormones, and their cellular receptors and fragments and mimetics thereof.

13. The method of Claim 12, wherein said sensitivity is continuously adjustable on a dose-responsive basis, including on a plurally stepped dose-responsive basis.

14. The method of Claim 12, wherein said sensitivity is discontinuously adjustable.

15. The method of Claim 12, wherein said sensitivity is adjustable both discontinuously and continuously.

16. The method of Claim 12, wherein an agent capable of interfering with function of the modulatory agent is added to regulate the relative or absolute amounts of the first or second interacting molecules.

17. The method of Claim 12, wherein the host cell is from a *Saccharomyces cerevisiae* strain containing:

three integrated reporters for the detection of two-hybrid interactions, the first being a construct yielding a quantifiable product, the second and third being suitable when activated, for the rescue of nutrient auxotrophies;

the first interacting molecule is provided by

(a) introducing into the host cell a plasmid containing ampicillin or kanamycin resistance genes, a colE1 origin of replication and a DNA sequence encoding a first hybrid protein comprising a bait polypeptide and a Gal4pBD DNA binding domain the expression of which is controlled by an integrated estrogen-inducible promoter; then

(b) inducing expression of the first hybrid protein by incubating the host cell in an exogenous activator capable of activating inducing the promoter; and

the second interacting molecule is provided by

(a) introducing into the host cell a plasmid containing ampicillin or kanamycin resistance genes, a colE1 origin of replication and a DNA sequence encoding a second hybrid protein comprising a prey polypeptide derived from a library and the carboxyl-terminal end of the Gal4pAD transcriptional activation domain the expression of which is controlled by a rat glucocorticoid-inducible promoter; then

(b) inducing expression of the second hybrid protein by incubating the host cell in an exogenous activator capable of activating the promoter.

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